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Chlamydial pelvic infection in cats: a model for the study of human pelvic inflammatory disease

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SUMMARY The inoculation of feline keratoconjunctivitis agent *(Chlamydia psittaci)* directly into the oviducts of eight cats produced an acute disease that was characterised by hyperaemia of the tissue and pronounced polymorphonuclear leucocyte infiltration of the epithelium and subepithelial stroma. The lumens of the tubes contained exudates of desquamated epithelial cells and polymorphonuclear leucocytes. After about 30 days the disease subsided leaving chronic inflammation with the tissue infiltrated with both polymorphonuclear and mononuclear cells. Fimbrial scarring and formation of adhesions were apparent by 40 to 50 days after inoculation. Chlamydiae were isolated in McCoy cell cultures from most cats, in one for as long as 51 days after inoculation. Inclusions were seen in histological sections or smears of cells from the fimbriae of four of the eight cats. Six of the eight cats developed antibodies to feline keratoconjunctivitis agent, which were detectable as early as 12 days after inoculation.

To facilitate repeated examinations of the cats' fallopian tubes, techniques for laparoscopy in cats and for the collection of specimens while under laparoscopic examination were developed. The latter technique has since been applied successfully in man.

Introduction

Pelvic inflammatory disease (PID), or salpingitis, is common in women of childbearing age. Most cases are caused by ascending infection from the lower genital tract. *Neisseria gonorrhoeae,* chlamydiae, mycoplasmas, and anaerobic bacteria have been implicated as causative organisms.[1-3] *Chlamydia trachomatis* has been established as a major cause of non-gonococcal genital infection[4] and PID.[3 5-7]

Several animal models have been used to study the production of salpingitis by chlamydiae. Strains of *C trachomatis* isolated from patients with PID have been shown to infect the oviducts of grivet monkeys after direct inoculation of organisms into the lumen of the tube or into the uterine cavity,[8 9] *C trachomatis* has also been shown to provoke salpingitis in rabbits after direct inoculation, but the agent could not be reisolated from the oviducts of any of the rabbits inoculated.[10] The agent of guinea pig inclusion conjunctivitis, a *Chlamydia psittaci* organism, can infect the distal horn of the uterus in guinea pigs after direct inoculation of the organism,[11] and ascending infection from the lower genital tract was achieved in these animals after immunosuppression mediated by cyclophosphamide.[12] Salpingitis has recently been produced in mice inoculated with the mouse pneumonitis strain of *C trachomatis* direct into the ovarian bursa.[13]

In the study reported here we experimentally infected the oviducts of cats with the agent of feline keratoconjunctivitis (FKC), which causes both ocular and genital infections in cats.[14] To facilitate these investigations, we have developed techniques for laparoscopy in cats and for collecting specimens from the oviducts of animals under laparoscopic examination. The course of the disease was traced by microbiological, serological, cytological, and histological methods.

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Materials and methods

EXPERIMENTAL ANIMALS

British, short haired, laboratory bred cats were used. They were aged between nine months and two years and weighed 1·5 to 2·9 kg at the start of the experiments.

CHLAMYDIAL INOCULUM

FKC agent, strain FKC/Ps/1/IOL-457/0, was used to infect the cats. This organism had originally been isolated from the eye of an infected cat, and had been passed three times in irradiated McCoy cells and three times in the yolk sacs of embryonated eggs. A yolk sac suspension containing about 100×10^9 inclusion forming units (ifu)/l was used as an inoculum, and was stored at $-70°C$ until used.

INOCULATION AND EXAMINATION OF CATS

All procedures were carried out under general anaesthetic induced by intramuscular and subsequent intravenous administration of alphaxalone and alphadone acetate (Saffan; Glaxo Laboratories, Greenford, Middlesex). Each oviduct was inoculated with about 0·1 ml of FKC agent through a 3·5 inch long 20 gauge spinal needle under laparoscopic examination. The tubes were subsequently examined by either laparotomy or laparoscopy.

LAPAROTOMY

The abdomen and medial aspect of each thigh was shaved, and the abdominal skin was prepared with 5% chlorhexidine solution. A 4 cm longitudinal subumbilical midline incision was made, and the abdominal muscles were divided to open the peritoneal cavity. After examination, the peritoneum and anterior abdominal wall were closed in layers with No 2 chromic catgut and No 00 Dexon subcutaneous interrupted sutures.

LAPAROSCOPY

After the cat's abdominal cavity had been shaved, a Verres needle was inserted through a small superficial incision in the midline 3 cm above the pubic ramus. About 1·5 l nitrous oxide gas was passed through this needle to insufflate the abdominal cavity. A purse string suture around the needle prevented an escape of gas. The operating table was then put into the Trendelenberg position with the cat's head downwards so that its intestines were out of view. A 7 mm trochar and appropriate cannula were inserted in the subumbilical midline area, an escape of gas once again being prevented by a purse string suture. A Storz 7 mm laparoscope was inserted through this cannula to enable the oviducts and ovaries to be viewed. Specimens for chlamydial isolation, differential cytology, local antibodies, and fimbrial biopsies were taken through another 2·8 mm cannula inserted into the abdominal cavity through an incision in the right lower abdominal quadrant.

When the examination was completed, gas was allowed to escape through the cannulae, which were then removed, and the subumbilical incision was closed with No 1 subcuticular Dexon sutures.

ISOLATION OF CHLAMYDIAE

Specimens for chlamydial isolation were collected from the fimbriae during laparotomy using dry, sterile, cotton wool swabs. During laparoscopy, specimens were collected using either a metal curette or a cytology brush (No BT/2·3/94/140 supplied by Meditech Inc, Watertown, Massachusetts, USA). All specimens were placed into 2SP (sucrose phosphate) transport medium[15] with additional 3% fetal bovine serum. In the case of the cytology brush, the last 1 cm of the plastic sheath was cut off into the transport medium after the brush had been withdrawn inside the sheath, thereby depositing collected material on to it. Specimens were stored at $-70°C$ until inoculated on to cycloheximide treated McCoy cell monolayers.[16] After being incubated for 48 hours the monolayers were fixed in absolute methanol, stained in a mixture of 1% weight per volume methyl green (nine parts) and 0·1% weight per volume neutral red (one part),[17] and examined for the presence of FKC agent inclusions.

DIFFERENTIAL CYTOLOGY OF FIMBRIAL SMEARS

Material remaining on the cytology brush after collection of the specimen for chlamydial isolation was smeared on a microscope slide, fixed in absolute methanol, and stained with Giemsa. These smears were examined for the presence of chlamydial inclusions and inflammatory cells, the prevalence of polymorphonuclear leucocytes being expressed as the number per 100 epithelial cells.

ANTIBODIES TO CHLAMYDIAE

Samples of blood and local fimbrial secretions were collected by absorption into hydrophilic cellulose sponges that had been prepared to absorb 0·1 ml of fluid.[18] These were stored at $-20°C$ until tested for antibodies to chlamydiae by a microimmunofluorescence test[19] using fluorescein labelled anticat serum (Gibco: Bio-Cult Ltd, Paisley, Scotland). Individual tests for IgG and IgM antibodies were not performed as suitable fluorescence conjugates were not available. Rhodamine labelled bovine serum albumin (Difco, West Molesey, Surrey) was used as a counterstain.

HISTOLOGY

Fimbrial biopsy specimens were collected using laryngeal microbiopsy forceps with a 1·8 mm diameter cup, after which the biopsy area was washed with a solution containing 500 IU/ml heparin to reduce any subsequent formation of adhesions. At the end of the experiments, a hysterectomy and bilateral salpingo-oophorectomy was performed, and the removed organs were

subjected to histological examination. Fixation was in 10% buffered formol saline, and sections were stained with either haematoxylin and eosin or Giemsa.

EXPERIMENTAL DESIGN

Experiment 1

To investigate the susceptibility of the cat oviduct to infection with FKC agent, two cats (cats 1 and 2) were inoculated with the organism direct on to the fimbriae while under laparoscopic examination. The cats were subsequently examined by laparotomy; cat 1 was examined on days 4 and 12 after inoculation and cat 2 on days 8 and 18.

After the first laparotomy operations, on days 4 and 8 respectively, both the cats in this experiment unfortunately developed superficial wound infections around their longitudinal midline incisions because the cats had eaten through individual subcuticular sutures, which made interpretation of results difficult.

Experiment 2

To avoid the problem of wound infection and to allow for repeated examinations of the oviducts over longer periods, we decided to make all further examinations using laparoscopy and to develop the techniques necessary for the examination and collection of specimens by this means. Three cats (cats 3, 4, and 5) were inoculated under laparoscopic examination as in experiment 1. To provide a range of observations covering as much of the infection as possible, examinations were staged as follows: cat 3 was examined on days 6, 30, 61, and 71 after inoculation; cat 4 was examined on days 4, 12, 52, and 55; and cat 5 was examined on days 7, 16, 43, and 55. No postoperative wound infections occurred in this group.

As examination of the oviducts in this experiment was by laparoscopy, specimens for chlamydial isolation could not be collected using cotton wool swabs as in experiment 1, because these could not be passed through the cannula that was used for the collection of specimens. A metal curette was therefore used to try to scrape material from the fimbriae for chlamydial culture, but this method of specimen collection proved to be unsatisfactory.

Experiment 3

Because of the poor results achieved using a metal curette to collect specimens for chlamydial isolation, other methods of collecting specimens under laparoscopic examination were investigated. Collection by means of a cytology brush appeared to be satisfactory, and a further three cats (cats 6, 7, and 8) were inoculated with FKC agent to investigate this method. Cats 6 and 7 were examined on days 4, 13, 20, 27, 40, and 51, and cat 8 on days 6, 13, 20, 40, and 51.

In addition, one cat (cat 9) was inoculated with an uninfected yolk sac preparation as a control and examined in the same way as the cats in experiment 3 on days 14, 21, and 35 after inoculation, with the cytology brush being used to collect specimens from the fimbriae on those days.

Results

GENERAL SIGNS

Cats 3 and 4 (in experiment 2) showed a slight disturbance, with general lethargy, anorexia, and slight weight loss on days 3 and 4 after inoculation; but by day 6 both were quite well, had good appetites, and had regained the weight they had lost. None of the other cats were affected in any obvious systemic manner apart from cats 1 and 2 that developed wound infections.

MACROSCOPIC APPEARANCE OF THE OVIDUCTS

Table I shows the pathological changes in the oviducts after inoculation. Evidence of inflammation, either hyperaemia or exudate, was seen in all cats except cat 2 during the first three weeks. Varying degrees of scar and adhesion formation were seen at later times. None of the cats showed any macroscopic signs of liver adhesions at necroscopy.

TABLE I *Presence of clinical signs on fallopian tubes of inoculated cats*

Days after inoculation	*Experiment 1*		*Experiment 2*			*Experiment 3*		
	Cat 1	*Cat 2*	*Cat 3*	*Cat 4*	*Cat 5*	*Cat 6*	*Cat 7*	*Cat 8*
4	H			H		–	–	
6			H					H
7					–			
8		–						
12	H			HE				
13						HE	H	HE
16					HE			
18		–						
20						H	H	–
27						–		
30			–					
40						S	S	SA
43					–			
51						S	S	A
52				S				
55				S				
61			–					
71			–					

H = hyperaemic fimbriae; E = exudate; S = scarring; A = adhesions; – = no abnormality detected.

ISOLATION OF CHLAMYDIAE

Chlamydiae were not isolated from the oviducts of any cat before inoculation. Table II shows that they were isolated from all the cats in experiments 1 and 3 after inoculation. Positive cultures were obtained

from cat 6 for as long as 51 days after inoculation. In experiment 2 chlamydiae were isolated from only one cat, cat 4, on day 4. The failure to isolate chlamydiae from the cats in this experiment may have been because using a metal curette was an unsatisfactory method of collecting specimens.

TABLE II *Isolation of chlamydiae from fimbriae of inoculated cats*

Days after inoculation	Experiment 1		Experiment 2			Experiment 3		
	Cat 1	Cat 2	Cat 3	Cat 4	Cat 5	Cat 6	Cat 7	Cat 8
4	+			+		+	−	
6			−					−
7					−			
8		+						
12	+			−				
13						+	+	+
16					−			
18		+						
20						+	+	+
27						−	−	
30			−					
40						+	−	−
43					−			
51						+	−	−
52				−				
55				−	−			−
61			−					
71			−					

+ = chlamydiae isolated; − = chlamydiae not isolated.

HAEMATOLOGICAL FINDINGS

In all the animals, both the white cell counts and the erythrocyte sedimentation rates varied with little relevance to the severity of the disease. The white cell counts ranged from 5500 to 14 500 before inoculation and from 6200 to 25 800 after inoculation. Table III shows that the erythrocyte sedimentation rates were generally raised in blood samples taken after inoculation, but they did not clearly correlate with the microscopic or macroscopic findings.

TABLE III *Erythrocyte sedimentation rates (mm in the first hour) in inoculated cats*

Days after inoculation	Experiment 1		Experiment 2			Experiment 3		
	Cat 1	Cat 2	Cat 3	Cat 4	Cat 5	Cat 6	Cat 7	Cat 8
0	2	2	15	18	23	1	3	2
4	4			55		30	2	
6			38					5
7					33			
8		5						
12	11							
13						45	11	1
16					54			
18		65						
20						6	2	11
27						32	3	
40						23	1	2
43					15			
51						23	2	3

HISTOLOGY

Histological sections taken from cat 9, which was inoculated with non-infected yolk sac material, showed no evidence of tubal inflammation at any time (fig 1).

Evidence of acute inflammation was seen in sections from the oviducts of infected cats taken as early as four days after inoculation. Blood vessels were dilated and there was an acute polymorphonuclear leucocyte infiltration into the epithelium and into the subepithelial stroma. Exudates of desquamated epithelial cells and polymorphonuclear leucocytes were seen in the lumens of the tubes (fig 2). Chlamydial inclusions were seen in sections from the fimbrial biopsy specimen taken from cat 3 on day 6 (fig 3). By day 16, the infiltration of the tissue consisted of a mixture of polymorphonuclear leucocytes and mononuclear cells (fig 4). Sections taken from the fimbriae between 51 and 55 days after inoculation showed evidence of chronic inflammation, with the formation of adhesions between fimbrial ridges (fig 5).

DIFFERENTIAL CYTOLOGY

Few cells, consisting almost entirely of epithelial cells, were collected on smears from the fimbriae of cat 9, which had been inoculated with a normal yolk sac suspension. Smears taken from infected cats, however, contained large numbers of epithelial cells, polymorphonuclear leucocytes, and mononuclear cells. The polymorphonuclear leucocytes in particular appeared to provide a convenient indicator of active disease, the results generally agreeing with the histological observations (table IV). Chlamydial inclusions were seen in smears collected from cat 6 on day 13 and from cat 8 on day 20 (fig 6).

TABLE IV *Prevalence* of polymorphonuclear leucocytes in fimbrial smears of inoculated cats*

Days after inoculation	Cat 6		Cat 7		Cat 8	
	Left	Right	Left	Right	Left	Right
4	1048	1159	32	68		
6					1	0
13	134	632†	2	8	2	4
20	756	236	1224	1127	385†	131
27	32	303	519	196		
40	4158	50	20	11	2	‡
51	17	‡	23	67	2	‡

*No of polymorphonuclear leucocytes per 100 epithelial cells counted in 10 non-adjacent microscope fields.
†Chlamydial inclusions seen on smear.
‡Insufficient cells on smear.

ANTIBODIES TO CHLAMYDIAE

Antibodies to chlamydiae were detected in serum and fimbrial specimens collected after inoculation from

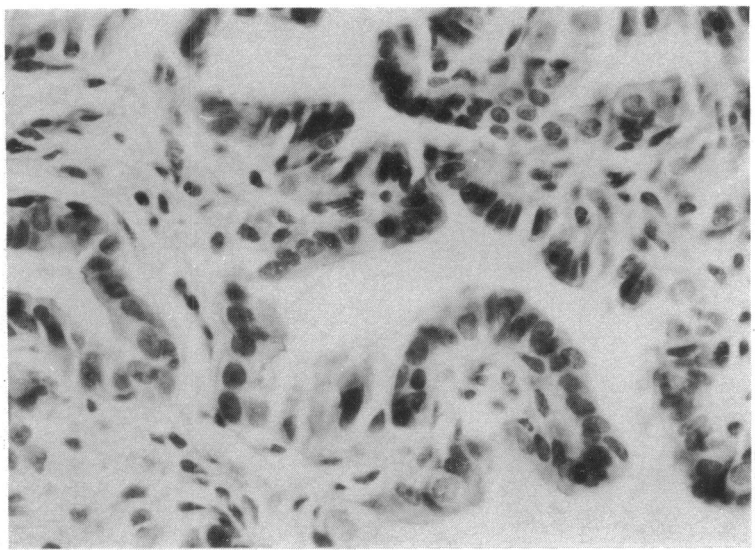

FIG 1 *Section through the fimbriae of cat 9 taken 35 days after inoculation with an uninfected yolk sac preparation. Haematoxylin and eosin × 600 (original magnification).*

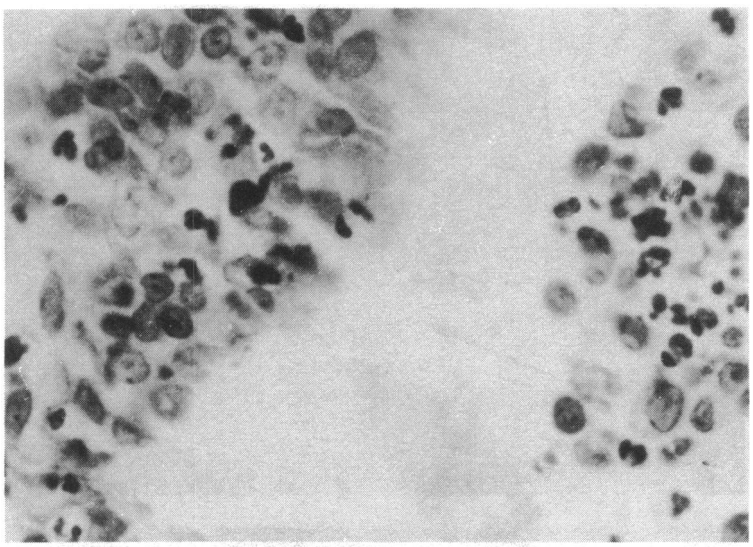

FIG 2 *Section of a fimbrial biopsy taken from cat 1 four days after inoculation with C psittaci showing polymorphonuclear leucocyte infiltration and exudate. Giemsa × 1200 (original magnification).*

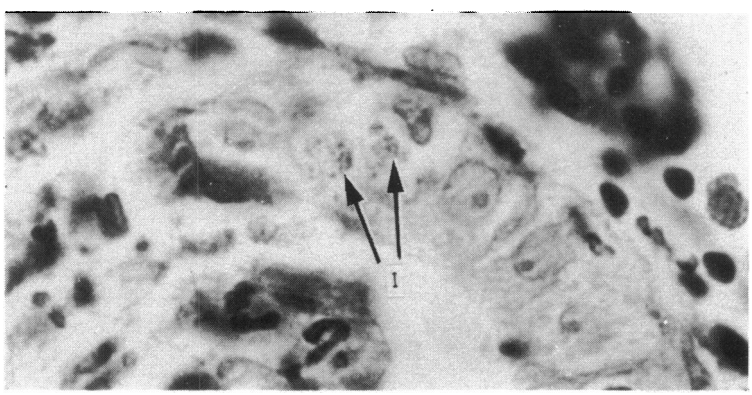

FIG 3 *Section from fimbriae of cat 3 taken on day 6 showing intracytoplasmic chlamydial inclusions (I). Giemsa × 1200 (original magnification).*

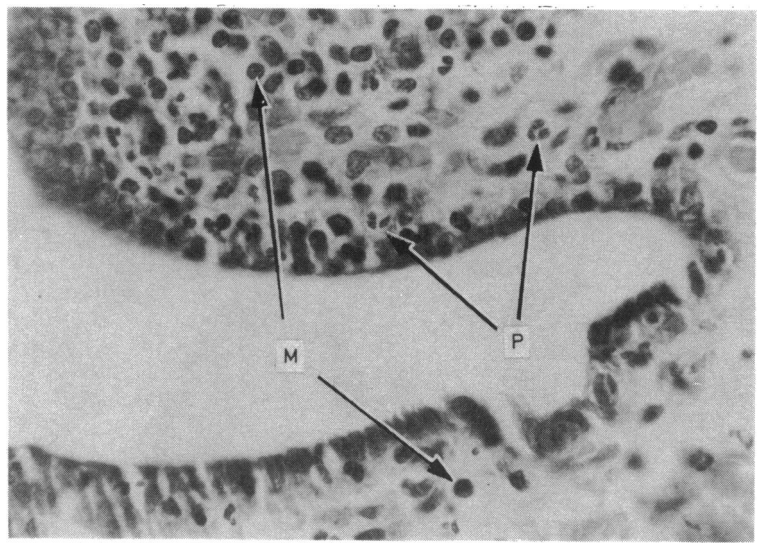

FIG 4 *Section of fimbriae taken from cat 5 on day 16 showing a mixed polymorphonuclear cell (P) and mononuclear cell (M) infiltration of the subepithelial tissue. Giemsa×600 (original magnification).*

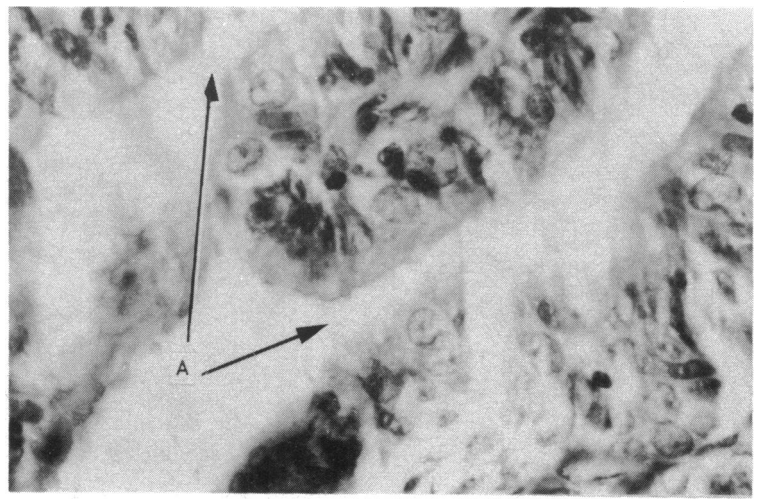

FIG 5 *Section through the fimbriae of cat 8 taken 51 days after inoculation, showing general inflammation, disruption of tissue, and adhesions (A) between fimbrial ridges. Giemsa×600 (original magnification).*

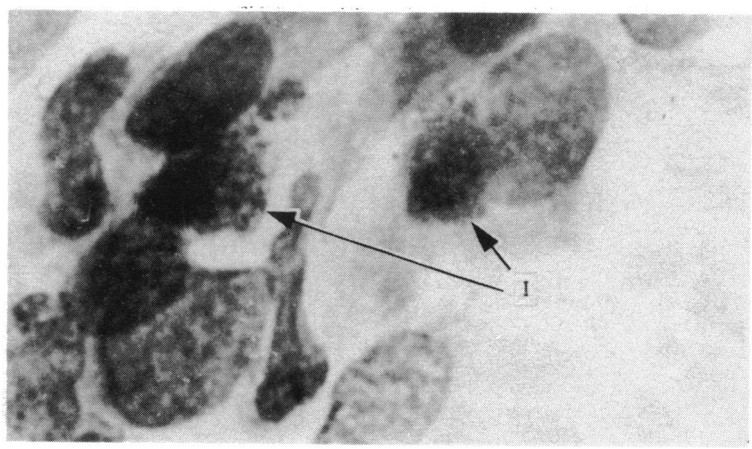

FIG 6 *Smear of cells collected from the fimbriae of cat 6 on day 13 showing two chlamydial inclusions (I). Giemsa ×1750 (original magnification).*

all infected cats except cats 4 and 5. Titres as high as 1/128 were detected as early as 12 days after inoculation and persisted at this level for up to 51 days in cats 7 and 8, and to 61 days in cat 3. No evidence of any decline in antibody titres was seen during this time. The antibodies were specific for FKC agent and did not cross react with a representative sample of other chlamydiae.

Discussion

The results of this study indicate that FKC agent can infect the oviducts of cats and produce a disease that consists of acute inflammation of the tubes, but little or no systemic disturbance of the animals. In spite of the mildness of the disease, considerable disruption of the infected tissues occurred, with the formation of fimbrial adhesions in the later stages of the disease.

In this study, the fimbriae were inoculated direct with chlamydiae to ensure that infectious organisms reached the oviducts. This method was used in preference to endocervical inoculation because of the presence of "valve" systems in the cat uterus and oviduct that we thought might inhibit the spread of infection from the cervix. Further experiments may clarify whether this is the case.

In previous studies of chlamydial PID in animals, the oviducts were examined either by repeated laparotomy or after killing the animal.[8-12] In this study we found that laparotomy, a major operation, is unsuitable for detailed studies of PID in cats because of a high risk of wound infection.

Laparoscopy is a minor operation that is used routinely in women to examine the fallopian tubes and ovaries. We adapted this procedure to examine the oviducts of cats, using a small 7 mm diameter laparoscope while the animal was firmly secured to a purpose built small animal operating table. Purse string sutures were needed around the cannulae and Verres needle to prevent the escape of gas from the abdomen. The operative wounds that resulted from this procedure were small and appeared to be much less prone to infection. Laparoscopy was carried out on the same animal up to seven times within two months without any complications.

Other workers have used either aspiration of the fallopian tubes[3] or tissue biopsies[8] to collect specimens for chlamydial culture. We thought that a method of collecting living cells from the epithelium should be more sensitive than aspiration for detecting chlamydiae, as aspiration would be expected to collect only desquamated cells and extracellular fluids. In a pilot study (unpublished observation) microbiopsy of the fimbriae resulted in scarring and formation of adhesions. This method was therefore considered to be unsuitable for repeated sampling of the same tissue. The cytology brush used for the cats studied in experiment 3 proved to be suitable for collecting specimens for chlamydial culture. The procedure was relatively painless and produced no pathological reactions when used to collect specimens from a non-infected cat. The brush head was protected by the plastic sheath during its passage through the valve in the cannula, and the collected material that was deposited on the inside of the sheath was used to inoculate cell cultures. This brush method had the added advantage of providing a specimen that could be used for differential cytology and to detect inflammatory cells and chlamydial inclusions. Since the completion of this study, similar brushes have been used to collect specimens for chlamydial culture and for differential cytology from human patients during laparoscopy.

During this investigation, biopsy specimens were taken for histology only once from each oviduct. To minimise the risks of scarring and the formation of adhesions the oviducts were immediately washed with a heparin solution.

The pathological reactions seen in the infected oviducts showed a noticeable similarity to those seen during chlamydial eye infections. As in guinea pig inclusion conjunctivitis,[20] initial polymorphonuclear leucocyte infiltration was followed after several weeks by mononuclear cell infiltration. As in the case of eye infections,[21] the tissue damage that resulted from the disease may have been associated with this mononuclear cell reaction.

The considerable disruption of the fimbriae and formation of adhesions that occurred as a result of the chlamydial infection may have been caused in part by the large size of the inoculum used. If similar processes were found to occur during *C trachomatis* infections in man, however, they might have considerable importance in the future fertility of the patient. The mild systemic reactions seen in the infected cats, despite the virulence of FKC agent for the eyes and lower genital tract of cats,[14] suggest that many cases of chlamydial PID in women may go unnoticed or undiagnosed.

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